

Norepinephrine depleting toxin DSP-4 and LPS alter gut microbiota and induce neurotoxicity in α -synuclein mutant mice

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Running Title: Gut microbiota in LPS/DSP-4 treated SNCA mice

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Supplemental Table 1. primer sequences for qPCR

Gene access #	Forward	Reverse
HM453208	GTCATTCCTCGGTGGATT	TTCATGTCATGTCGTCCAT
HM453207	TGACCATGTCGTTGCCTGT	TTTCATGGACCCCTTCTC
AJ400275 (4)	ATCATTACAATGGGGAAA	GGCACAGAGTTAGCCGTCTC
NZ_CCDQ010000017	GCTGTTCCATAGGGGGAGTA	AAACCAGTGGCAACAAAGGTC
AY271254	ATCATTACAATGGGGAAA	GGCACAGAGTTAGCCGTCTC
EU728784	CGAAAGGTTAGCGGTGAAG	CGTAGGAGTTGGACCGTGT
Prevotella (2)	CATGACGTTACCCGCAGAAGAAG	TCCTGCACGCTACTTGGCTG
M31663	GACACGTGTACCCAATGCAG	GGCAACCTGAGCCTCTGTAG
NR_041684	GCAGCAGTTGAACAACCAGA	ATGCGTATGCCCTTTGGTC
MIB (3)	CCAGCAGCCGCGGTAAATA	CGCATTCCGCATACTTCTC
Bact (3)	GGTTCTGAGAGGAGGTCCC	GTCGCCTCCCGTAGGAGT
Bact (2)	GGARCATGTGGTTAACCGATGAT	AGCTGACGACAACCATGCAG
Prevotella (1)	GGTGTGGCTTAAGTGCAT	CGGACGTAAGGGCCGTGC
AJ400264 (4)	GAACCCGAAAACCTGTCTCA	TTTACCCTAGGGCGTTCCCT
KP076661	ACACGGACCAGACTCCTACG	ACACGTCCCGCACTTATT
926-1040 Firm (1-2)	GGAGYATGTGGTTAACCGAAGCA	AGCTGACGACAACCATGCAC
M59090	AGTAACCGCGTGGGTAACCTG	ACCGGAGTTTCACACCAG
AY487161	GGGTCAATTGCAGGAAAGAA	GTTGATGGAGCTGGTCAGT
KX247777	TTTGAGTGAATGGCGAACTG	CCAAAAGTGTAGCCGAAGC
HM079525	CCCAAGAGATCGGGATAACA	AGGCCGTTACCCCTACCAA
Lact (3)	AGCAGTAGGAAATCTCCA	CACCGCTACACATGGAG
SFB (3)	GACGCTGAGGCATGAGAGCAT	GACGGCACGGATTGTTATTCA
X73441	AGGATCGTGAATTCTGTG	CGGCCTGGACTACTAGGGTA
AJ400237 (4)	GGTAAACGGCTTACCAAGTCA	GTGCAATTCCCCACTGCT
EF025906	CGCAGCAAACGCAATAAGTA	TAAGGTTCTCGCGTTGCTT
U13039	TAACGGCTTACCAAGGCAAC	GTGCAATTCCCCACTGCT
U67159	CCCTTATGACCTGGCTACA	CAATCCGAACTGGGATCTGT
HQ326029	ACCAGTACCAACCTGCCAAAG	TGGTGCCTTGCTTAAACCT
KU321269	ATATTGCACAATGGGGAAA	AGCCGGAGCTTCCTCCTAG
FBXY01000003	TGCTCCAAGCAGCTCTACAA	CTGCCGTATTGGAAAACAT
LN999030	GGAACAGCCGTACAGGGTA	GAACACGAAGAACGCCAAAT
Cleft (3)	GTTGACAAAACGGAGGAAGG	GACGGGCGGTGTACCAA
MF186848	GTGAGGAACCTGCCTCAAAG	GGCCGTTACCCACCAA
Act920F3 (3)	TACGGCCGCAAGCTA	TCRTCCCCACCTCCCTCG
KP076663	AGCTTGTGGTGGGTAATG	GTGCAATTCCCCACTGCT
DQ672261	TGGGGTAGTGGCTTACCAAG	GTGCAATTCCCCACTGCT
AY488182	AGGGTCTGAAGAACGTGGT	TTGCAGCAATTGTTCTTG
KY194703	TCGGGGTGAGTGTACCTTC	GCCCTTACGCCAATAAAAT
NR113172	GCCCCGTCAAGTCATGAAAGT	CCGGTACGGCTACCTGT
Eco1457F (1)	CATTGACGTTACCCGCAGAAGAAGC	CTCTACGAGACTCAAGCTTGC
DQ836271	GCATCACTGATCCCATTCT	TGAGTCACCGCTACAGCAC
AFOF01000027	CGAGTCGATGTCAGTGTG	TCAGCTACACCAACGGTGAC
KF358344	AGCCACATTGGGACTGAGAC	CAACCCCTAAGGCCCTCATCA
AF052586	AGAACTCCAGGTGCCCTCTT	AGAAGCAGCGTTGGTGTAT
AY324832	GCGGTAAGCCTGAAAACAG	CTCATGACGAGGGTTCGATT
EU285109	GAGGAATTACCCAGGTGCT	GTGATGGGTTGGCTTGT
U02514	GATTGTCGCTTCCAGCTC	GTCCGCAACCGTTGAGTAAT
NZ_CCXU01000080	CGAGTTAACCAAGTCCCAAGC	CATTAGGACAGGGTGCAGGT
NZ_KQ235732	GGAGCCACTATCGACTACGC	GTCCTGATGGTCGTACATCT
Eub338-518 (1-3)	ACTCCTACGGGAGGAGCAG	ATTACCGCGGCTGCTGG

(1) Medina et al., 2017; (2) Uebenso et al., 2017, (3) Wellman et al., 2017, (4) Zhang et al., 2014

Supplementary Table 2. Relative expression of 16S rRNA in the "Two-hit" mouse models

Phylum	Family	Gene access #	WT littermate			SNCA		
			Cont	LPS	DSP4	Cont	LPS	DSP4
Verrucomicrobia	Verrucomicrobiaceae	HM453208	100 ± 53	1100 ± 412	947 ± 422	760 ± 386	2410 ± 615	2930 ± 1040
	Verrucomicrobiaceae	HM453207	100 ± 15	170 ± 63	83 ± 27	107 ± 14	236 ± 58	154 ± 38
	Verrucomicrobiaceae	AJ400275 (4)	100 ± 34	977 ± 466	134 ± 86	348 ± 162	67 ± 53	106 ± 40
	Akkermansiaceae	NZ_CCDQ010000017	100 ± 47	1714 ± 735	321 ± 200	865 ± 331	153 ± 111	283 ± 115
	Akkermansiaceae	AY271254	100 ± 55	937 ± 492	93 ± 79	195 ± 104	58 ± 52	54 ± 18
Bacteroidetes	Prevotellaceae	EU728784	100 ± 13	53 ± 5	41 ± 14	23 ± 5	4 ± 0.5	6 ± 1
	Prevotellaceae	Prevotella (2)	100 ± 18	86 ± 4	103 ± 24	115 ± 15	97 ± 7	61 ± 9
	Prevotellaceae	NR_041684	100 ± 34	61 ± 7	94 ± 24	51 ± 11	5 ± 1	6 ± 2
	Bacteroidaceae	M31663	100 ± 26	63 ± 17	110 ± 42	51 ± 14	13 ± 2	16 ± 4
	Bacteroidaceae	MIB (3)	100 ± 20	72 ± 11	97 ± 32	110 ± 33	65 ± 15	193 ± 52
	Bacteroidaceae	Bact (3)	100 ± 28	80 ± 11	72 ± 18	122 ± 37	57 ± 9	94 ± 16
	Bacteroidaceae	Bact (2)	100 ± 12	106 ± 6	93 ± 19	144 ± 27	64 ± 8	83 ± 10
	Porphyromonadaceae	Prevotella (1)	100 ± 9	103 ± 9	98 ± 14	112 ± 15	77 ± 6	133 ± 10
	Porphyromonadaceae	AJ400264 (4)	100 ± 25	61 ± 12	102 ± 39	101 ± 20	34 ± 9	70 ± 24
Firmicutes	Tannerellaceae	KP076661	100 ± 16	125 ± 30	101 ± 12	160 ± 15	73 ± 15	127 ± 26
	Firmicutes	926-1040 Firm (1-2)	100 ± 28	58 ± 5	62 ± 12	88 ± 15	74 ± 8	56 ± 7
	Lachnospiraceae	M59090	100 ± 11	54 ± 6	59 ± 17	55 ± 4	61 ± 20	45 ± 15
	Lactobacillaceae	AY487161	100 ± 45	84 ± 38	74 ± 9	32 ± 8	17 ± 7	11 ± 2
	Lactobacillaceae	KX247777	100 ± 26	154 ± 61	71 ± 9	105 ± 16	121 ± 60	77 ± 11
	Lactobacillaceae	HM079525	100 ± 18	52 ± 16	50 ± 19	20 ± 7	3 ± 1	8 ± 2
	Lactobacillaceae	Lact (3)	100 ± 17	76 ± 22	53 ± 16	82 ± 17	117 ± 31	119 ± 42
	Clostridiaceae	SFB (3)	100 ± 35	53 ± 28	35 ± 21	184 ± 88	126 ± 16	72 ± 43
	Erysipelotrichaceae	X73441	100 ± 39	41 ± 19	25 ± 10	24 ± 19	67 ± 19	104 ± 25
	Peptostreptococcaceae	AJ400237 (4)	100 ± 20	86 ± 13	83 ± 16	162 ± 39	115 ± 19	158 ± 31
	Lachnospiraceae	EF025906	100 ± 28	58 ± 7	88 ± 29	105 ± 18	83 ± 9	59 ± 8
	Eubacteriaceae	U13039	100 ± 23	75 ± 15	110 ± 25	151 ± 21	111 ± 16	95 ± 12
	Eubacteriaceae	U67159	100 ± 21	96 ± 17	95 ± 27	245 ± 35	213 ± 29	221 ± 19
	Staphylococcaceae	HQ326029	100 ± 18	91 ± 11	104 ± 15	107 ± 22	52 ± 9	15 ± 2
	Ruminococcaceae	KU321269	100 ± 34	61 ± 7	94 ± 24	51 ± 11	5 ± 1	6 ± 1
	Ruminococcaceae	FBXY01000003	100 ± 48	53 ± 13	44 ± 9	51 ± 8	42 ± 9	25 ± 6
	Ruminococcaceae	LN999030	100 ± 58	89 ± 34	16 ± 15	33 ± 21	33 ± 21	6 ± 6
	Ruminococcaceae	Cleft (3)	100 ± 30	50 ± 10	70 ± 13	82 ± 9	71 ± 11	52 ± 4
	Ruminococcaceae	MF186848	100 ± 27	96 ± 34	103 ± 34	126 ± 43	147 ± 31	91 ± 16
Actinobacteria	Actinobacterias	Act920F3 (3)	100 ± 17	90 ± 17	146 ± 28	43 ± 11	36 ± 7	23 ± 5
	Promicromonasporaceae	KP076663	100 ± 26	68 ± 20	53 ± 16	61 ± 13	44 ± 5	25 ± 6
	Propionibacteriaceae	DQ672261	100 ± 36	82 ± 14	93 ± 11	137 ± 35	35 ± 12	5.1 ± 3.4
	Bifidobacteriaceae	AY488182	100 ± 35	56 ± 19	91 ± 32	41 ± 9	54 ± 1	16 ± 7
	Bifidobacteriaceae	KY194703	100 ± 35	74 ± 24	93 ± 26	77 ± 30	94 ± 19	83 ± 29
	Bifidobacteriaceae	NR113172	100 ± 37	58 ± 13	67 ± 20	88 ± 14	87 ± 17	77 ± 5
Proteobacteria	Enterobacteriaceae	Eco1457F (1)	100 ± 60	848 ± 277	1034 ± 666	1270 ± 416	1513 ± 609	2208 ± 773
	Morganellaceae	DQ836271	100 ± 25	82 ± 16	143 ± 37	221 ± 38	153 ± 64	131 ± 60
	Bradyrhizobiaceae	AFOF01000027	100 ± 34	55 ± 10	130 ± 45	55 ± 12	60 ± 19	85 ± 31
	Bradyrhizobiaceae	KF358344	100 ± 30	50 ± 17	110 ± 25	43 ± 5	35 ± 4	56 ± 15
	Pseudomonadaceae	AF052586	100 ± 28	40 ± 13	100 ± 28	143 ± 55	59 ± 17	38 ± 12
	Pseudomonadaceae	AY324832	100 ± 29	33 ± 7	54 ± 14	74 ± 13	39 ± 4	22 ± 6
	Xanthomonadaceae	EU285109	100 ± 25	41 ± 7	40 ± 7	108 ± 32	36 ± 5	23 ± 4
	Enterobacteriaceae	U02514	100 ± 28	228 ± 99	206 ± 97	427 ± 122	91 ± 27	94 ± 27
Euryarchaeota	Methanobacteriaceae	NZ_CCXU01000080	100 ± 27	54 ± 5	111 ± 33	72 ± 13	78 ± 6	84 ± 14
Fusobacteria	Fusobacteriaceae	NZ_KQ235732	100 ± 34	84 ± 11	90 ± 15	104 ± 27	66 ± 6	115 ± 20

Data are mean ± SE of 4-7 animals. (1) Medina et al., 2017; (2) Uebenso et al., 2017; (3) Wellman et al., 2017; (4) Zhang et al., 2014

Supplementary Table 3: Firmicutes/Bacteroids ratio

Phylum	Family	Gene access	WT littermate			SNCA		
			Cont	LPS	DSP4	Cont	LPS	DSP4
Bacteroidetes	Prevotellaceae	EU728784	100	53	41	23	4	6
	Prevotellaceae	Prevotella	100	86	103	115	97	61
	Prevotellaceae	NR_041684	100	61	94	51	5	6
	Bacteroidaceae	M31663	100	63	110	51	13	16
	Bacteroidaceae	MIB (1)	100	72	97	110	65	193
	Bacteroidaceae	Bact (1)	100	80	72	122	57	94
	Bacteroidaceae	Bact (2)	100	106	93	144	64	83
	Porphyromonadaceae	Prevotella	100	103	98	112	77	133
	Porphyromonadaceae	AJ400264	100	61	102	101	34	70
	Tannerellaceae	KP076661	100	125	101	160	73	127
Firmicutes	Firmicutes	926-1040 Firm	100	58	62	88	74	56
	Lachnospiraceae	M59090	100	54	59	55	61	45
	Lactobacillaceae	AY487161	100	84	74	32	17	11
	Lactobacillaceae	KX247777	100	154	71	105	121	77
	Lactobacillaceae	HM079525	100	52	50	20	3	8
	Lactobacillaceae	FJ462287	100	76	53	82	117	119
	Clostridiaceae	SFB	100	53	35	184	126	72
	Erysipelotrichaceae	X73441	100	41	25	24	67	104
	Peptostreptococcaceae	AJ400237	100	86	83	162	115	158
	Lachnospiraceae	EF025906	100	58	88	105	83	59
	Eubacteriaceae	U13039	100	75	110	151	111	95
	Eubacteriaceae	U67159	100	96	95	245	213	221
	Staphylococcaceae	HQ326029	100	91	104	107	52	15
	Ruminococcaceae	KU321269	100	61	94	51	5	6
	Ruminococcaceae	FBXY01000003	100	53	44	51	42	25
	Ruminococcaceae	LN999030	100	89	16	33	33	6
	Ruminococcaceae	Cleft (2)	100	50	70	82	71	52
	Ruminococcaceae	MF186848	100	96	103	126	147	91
			Firm	73.7222	68.667	94.611	81	67.778
			Bact	81	91.1	98.9	48.9	78.9
			Ratio	0.91015	0.7538	0.9566	1.6564	0.859